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Contribution of diatoms as food sources for post-larval abalone *Haliotis discus hannai* on a crustose coralline alga

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Abstract

Survival and growth rates of post-larval abalone *Haliotis discus hannai* reared on a crustose (non-geniculate) coralline alga (CCA) *Lithophyllum yessoense* with diatoms (CCA + diatoms) and without diatoms (CCA – diatoms) were compared in the laboratory in order to determine the contribution of diatoms on CCA as food sources for post-larvae and the dietary value of CCA themselves. Experiments were performed for 5 weeks with two groups of different developmental stage, newly metamorphosed stage (younger post-larvae) and over 1 mm stage (older post-larvae). Both stages of post-larvae reared on CCA + diatoms grew well (53.4 ± 2.4 μm/day; mean ± SE and 85.0 ± 4.0 μm/day; mean ± SE, respectively). Younger post-larvae grew to over 2 mm in 5 weeks, and older ones reached over 4 mm in 5 weeks. There was no difference in shell length of younger post-larvae reared on CCA + diatom and CCA – diatom at the week 1. However, the mean growth rate of abalone on CCA – diatoms from the second to fifth week (24.4 ± 1.9 μm/day; mean ± SE) was significantly lower than that of individuals on CCA + diatoms and the younger post-larvae on CCA – diatoms reached only 1 mm shell length in 5 weeks. The older post-larvae also did not grow well (27.8 ± 3.3 μm/day; mean ± SE) on CCA – diatoms and did not reach 3 mm by the end of the experiment. On CCA + diatoms, 74.6 ± 2.0 % (mean ± SE) of the younger post-larvae survived for the 5 weeks experiment. In contrast, the survival rate of the younger post-larvae reared on CCA – diatoms decreased rapidly until the second week, and 25.0 ± 12.6% (mean ± SE) of the individuals survived by the fifth week. The results of this study indicate that diatoms are essential for the rapid growth of post-larval abalone on the CCA. It is considered that the CCA *L. yessoense* itself is not a principal food source for post-larval *H. discus hannai* from approximately 500 μm to at least 3 mm shell length.

Introduction

The planktonic larvae of several species of the abalone genus *Haliotis* preferentially settle on crustose (non-geniculate) coralline algae (CCA) (Saito 1981; Morse and Morse 1984; Shepherd and Turner 1985; McShane and Smith 1988). At the time of metamorphosis, much of the yolk reserves is exhausted and post-larvae need to ingest an exogenous energy source immediately after metamorphosis (M. Asano and T. Kawamura 1992, unpublished). Thus CCA must provide food materials for the metamorphosed post-larvae. Some materials derived from CCA have been suggested to be possible food sources for post-larval abalone; surface mucus for *H. rufescens* Swaison 1822 (Morse and Morse 1984), cuticle and epithallial contents for *H. rubra* Leach 1814 (Garland *et al.* 1985) and outermost layer cells for *H. laevigata* Donovan 1808 and *H. scalaris* Leach 1814 (Shepherd and Daume 1996). Bacteria associated with CCA were suggested to be possible food

sources for *H. rubra* (Garland *et al.* 1985). Diatoms (Kawamura 1996) and trail mucus of juvenile and adult abalone (Takami *et al.* 1997) on CCA were also considered to be possible foods for *H. discus hannai* Ino 1953. Although it has been reported that juvenile *H. discus hannai* (Tomita and Tazawa 1971), *H. laevigata* and *H. scalaris* (Shepherd and Cannon 1988) from 5 to 10 mm in shell length feed directly on CCA themselves, the principal food sources for the post-larval abalone on CCA have not been elucidated yet.

In seed production hatcheries of abalone, benthic diatoms have been used as initial foods for post-larvae up to about 5 mm shell length. Recently the dietary value of benthic diatoms for the growth of post-larval abalone has been investigated in detail (Kawamura and Takami 1995; Kawamura *et al.* 1995). The results of these studies strongly suggest that benthic diatoms growing on CCA play important roles as food sources for post-larval abalone in the natural environment as well as in hatcheries (Kawamura *et al.* 1995; Kawamura 1996).

In the present study, survival and growth rates of post-larval abalone *H. discus hannai* reared on a CCA *Lithophyllum yessoense* Foslie 1909 with diatoms (CCA + diatoms) and without diatoms (CCA – diatoms) were compared in order to know the contribution of diatoms on CCA as food sources for post-larvae and the dietary value of CCA themselves.

Materials and Methods

Rocks encrusted with a CCA, *L. yessoense* were collected subtidally at Eno-shima Island, Miyagi Prefecture, Japan. The size of rocks was: 42.0–62.5 mm in length; 28.6–50.8 mm in width and 18.3–41.5 mm in breadth. The rocks were transported in a running seawater tank on a boat within 3 hours and kept in a running seawater tank at Tohoku National Fisheries Research Institute (TNFRI; Miyagi, Japan). The visible epibenthos, epiphytes and debris on the rocks except CCA were scraped off carefully, and rocks with CCA were rinsed well with filtered seawater (0.45 μm Millipore filter) to reduce diatoms and other micro organisms on CCA (rinsed CCA rocks) just before the experiments. All rocks used in the experiment were completely covered with CCA.

The experiment was carried out twice (Experiment 1: November 1995, Experiment 2: June 1995) using different sizes of abalone. In Experiment 1, growth and survival rates of post-larvae were compared for 5 weeks from just after metamorphosis between two experimental treatments; CCA + diatoms and CCA – diatoms. These treatments were set up as follows:

CCA + diatoms: Rinsed CCA rocks in sand-filtered seawater, which includes diatoms but not visible organisms, to encourage contamination with naturally occurring benthic diatoms.

CCA – diatoms: Rinsed CCA rocks used in filtered seawater (0.45 μm) with 6 mg/l concentration of GeO_2 (GeO_2 seawater).

GeO_2 seawater was prepared by the method of Chapman (1973). GeO_2 inhibits growth of diatoms, but does not affect the growth of any other algae (Chapman 1973).

Larval abalone used in Experiment 1 were hatched out in November 1995 at the Akita Prefectural Hatchery Center (Akita, Japan) according to the procedures described by Uki and Kikuchi (1984). Four days after fertilization at 20°C, the veliger larvae were transported to TNFRI within 5 hours. It was previously confirmed that the transport of veliger larvae by the method used in this study did not affect larval behaviour and development (Kawamura and Kikuchi 1992). The rinsed CCA rocks were placed on the bottom of 500 ml glass beakers with 300 ml of sand-filtered seawater (CCA + diatoms) or GeO_2 seawater (CCA – diatoms) and one rock per beaker. Fifty 5-day-old larvae, which were ready to metamorphose (Seki and Kan-no 1981), were placed in each beaker. Three replicates were used for each experimental treatment. Larvae were reared for 5 weeks at 20 °C, 6000 lux with a 12 hL: 12 hD photo-cycle. The shell length of individuals selected at random (5–7 inds. per beaker) was measured using an inverted optical microscope every week (week 1– 5). Survival rate of post-larvae was also assessed by counting the number of dead individuals during the experimental period. At the time of observation, all post-larvae were carefully removed from the CCA rocks using a water jet from a pipette and the rocks in beakers were replaced with new rinsed CCA rocks. Seawater in the

beakers was also changed. Another three rinsed CCA rocks just before the beginning of the rearing experiment and three old rocks each used in CCA + diatoms and CCA – diatoms were randomly selected and the diatoms on their surface were scraped off and fixed in a 5 % formalin-seawater solutions. Cell counts and identifications of diatoms were done under a light microscope. The surface areas of CCA rocks were calculated by approximating their shapes to spheres or polyhedron, and the benthic diatom cell density on the rocks was estimated. At the end of the experiment, we examined the gut contents of some live post-larvae reared on CCA + diatoms and CCA – diatoms.

In Experiment 2, growth rates of abalone from approximately 1.5 mm shell length were compared between CCA + diatoms and CCA – diatoms under the same environmental conditions as Experiment 1, except for the method of treatment of the rocks. The rocks used in Experiment 2 were previously soaked for 8 days in sand filtered (CCA + diatoms) or GeO_2 seawater (CCA – diatoms) before use. Larval abalone used in this experiment were hatched out in May 1995 at the Marine Development Company Ltd. (Iwate, Japan) using the same method as Experiment 1. Post-larvae were reared at the TNFRI under the CCA + diatoms condition until June 1995 when the experiment started. Seven individuals were reared in each experimental treatment. The shell length of all individuals was measured every week. Simultaneously, rocks and seawater were changed and diatom cells on the rocks were counted. Measuring and rearing methods were the same as in Experiment 1, but no replicates were conducted in Experiment 2. Gut contents of some live individual abalone on CCA + diatoms and CCA – diatoms were examined at the end of the experiment.

We evaluated the effects of GeO_2 on the rate of metamorphosis and peristomal shell formation and post-larval growth. These rates for individuals reared in GeO_2 seawater were compared with those for individuals reared in filtered seawater (0.45 μm). Metamorphosis and peristomal shell formation

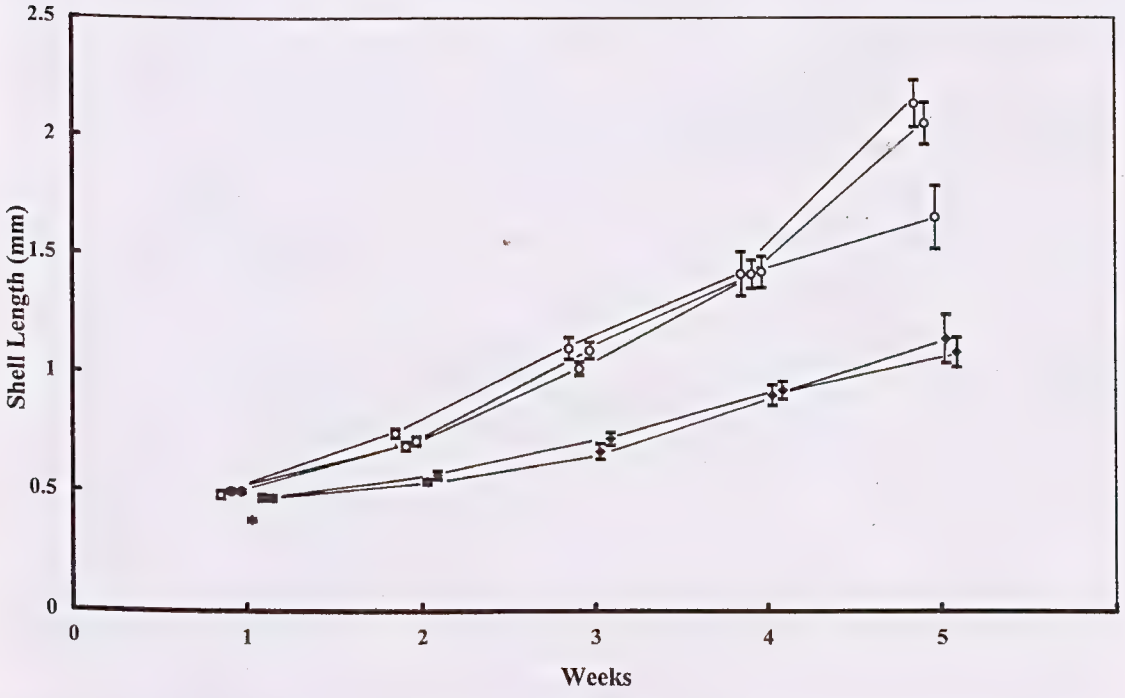

Figure 1. Growth of *Haliotis discus hannai* post-larvae (newly metamorphosed) fed on CCA + diatoms (open circle) and CCA – diatoms (closed diamond). Data are mean shell length of post-larvae (± SE, n = 5-7) on each rock.

rates were measured as follows. Three replicates were tested for each experimental treatment (filtered seawater and GeO_2 seawater) using rocks encrusted with *L. yessoense* as substrata for larval settlement. Rocks were placed on the bottom of 500 ml glass beakers with 300 ml filtered seawater or GeO_2 seawater. Fifty 5-day-old larvae (siblings of those used in Experiment 1) were placed in each beaker. Forty-eight hours after the introduction of larvae to the beakers, the number of individuals that had metamorphosed and formed peristomal shells was counted using an inverted microscope. The effect of GeO_2 on post-larval growth was also evaluated in three different size of post-larval abalone approximately 1 and 2 mm in shell length (siblings of Experiment 1 animals) and newly metamorphosed post-larvae (siblings of Experiment 2 animals). These individuals were reared in filtered seawater or GeO_2 seawater on the conditioned plates with suitable food sources such as trail mucus of adult abalone and benthic diatom *Cocconeis scutellum* var. *parva* (Grunow) Cleve 1895 for post-larval abalone (Takami *et al.* 1997). After 7 or 15 days from the initiation of rearing, the shell length of individuals was measured.

All statistical analyses were carried out with the JMP (SAS Institute Inc. 1994) statistical computer package. Statistical tests of settlement and survival rates were carried out on arcsine-transformed data. This transformation helped to normalize the data and reduce heteroscedasticity. Untransformed values are presented in graphs and tables.

Results

In Experiment 1, most veliger larvae (5 days old) settled on both CCA + diatoms and CCA – diatoms rocks within 24 hours. Mean settlement rates of 3 replicates were 99.1 and 99.3 % on CCA + diatoms and CCA – diatoms, respectively. Subsequently, the post-larvae reared on CCA + diatoms

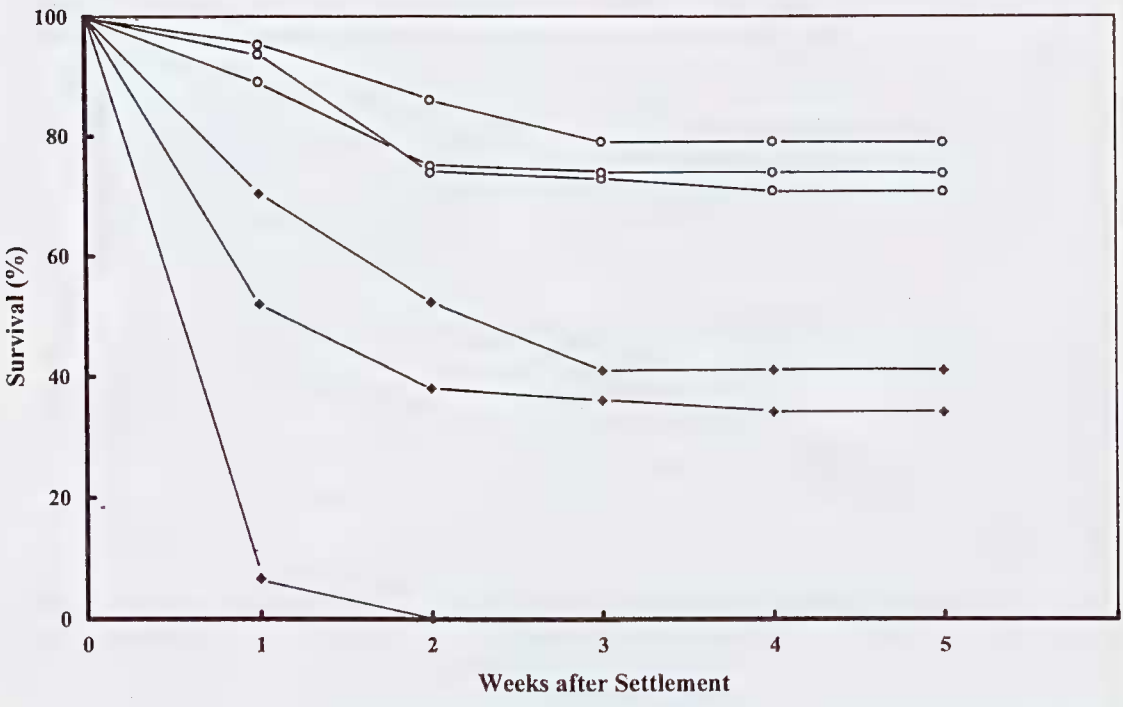

Figure 2. Survival of *Haliotis discus hannai* post-larvae reared on CCA + diatoms (open circle) and CCA – diatoms (closed diamond). Data show the proportion of 50 added post-larvae surviving for each CCA rock.

Table 1. The mean densities of benthic diatoms colonizing on rocks (n=3) encrusted with CCA *Lithophyllum yessoeuse* used in the Experiment 1.

Diatom species	No. of cells (cells/mm², mean ± SE)		
	Before experiment	After experiement	
		Sand filtered seawater	Filtered (0.45 μm) seawater dissolved with GeO_2
Achnanthes spp.	ND	5.1 ± 2.6	ND
Amphora spp.	70.7 ± 26.3	432.0 ± 69.3	37.3 ± 22.5
Cylindrotheca closterium	ND*	230.1 ± 36.0*	ND*
Cocconeis spp.	11.1 ± 1.1	48.9 ± 9.1	9.0 ± 3.3
Navicula spp.	15.3 ± 4.1	48.8 ± 10.2	11.4 ± 9.7

ND: not detected

* There is a possibility that these values include cells of *Nitzschia longissima*. It was difficult to identify *Cylindrotheca closterium* and *Nitzschia longissima* by light microscopic observation.

grew well and reached about 2 mm shell length (53.4 ± 2.4μm/day; mean ± SE, Fig. 1) after 5 weeks. The post-larvae reared on CCA - diatoms reached 1 mm shell length at the end of Experiment 1. There was no difference in shell length of post-larvae reared on CCA + diatoms (496.3 ± 13.8; mean ± SE) and CCA – diatom (473.4 ± 20.2; mean ± SE) at week 1. However, the mean growth rate of individuals reared on CCA – diatoms from week 2 to week 5 (24.4 ± 1.9μm/day; mean ± SE) was significantly lower than that of abalone reared on CCA + diatoms (t-test, $p<0.01$; Fig 1). On CCA + diatoms, 74.6 ± 2.0 % (mean ± SE) of the individuals survived throughout the experimental period. In contrast, the survival rate of the individuals reared on CCA – diatoms was significantly lower by the second week (t-test, $p<0.01$). Only 25.0 ± 12.6 % (mean ± SE) of the individuals survived by the end of the experiment (Fig 2).

Figure 3 shows the growth of post-larval abalone (> ca. 1.5 mm in shell length) reared on CCA + diatoms and CCA – diatoms in Experiment 2. On CCA + diatoms, individuals grew well during the

Table 2. The mean densities of benthic diatoms colonizing on rocks (n=3) encrusted with CCA *Lithophyllum yessoeuse* used in the Experiement 2.

Diatom species	No. of cells (cells/mm², mean ± SE)			
	Before experiment [a]		After experiement	
	Sand filtered seawater	Filtered (0.45 μm) seawater dissolved with GeO_2	Sand filtered seawater	Filtered (0.45 μm) seawater dissolved with GeO_2
Amphora spp.	214.3 ± 68.1	64.2 ± 24.1	141.3 ± 33.8	57.8 ± 15.8
Cocconeis spp.	59.7 ± 49.0	1.8 ± 1.8	42.3 ± 8.7	ND
Cylindrotheca closterium[b]	78.4 ± 41.6[b]	ND[b]	21.6 ± 6.5[b]	ND[b]
Navicula spp.	84.8 ± 40.1	ND	61.0 ± 27.2	ND
Nitzschia spp.	ND	ND	14.2 ± 14.2	ND
Melosira sp.	ND	ND	4.7 ± 4.7	ND

ND: not detected

[a] Rocks were stocked in sand filtered seawater or filtered seawater dissolved with GeO_2 for 8 days before use of experiment in Experiment 2.

[b] There is a possibility that these values include cells of *Nitzschia longissima*. It was difficult to identify *Cylindrotheca closterium* and *Nitzschia longissima* by light microscopic observation.

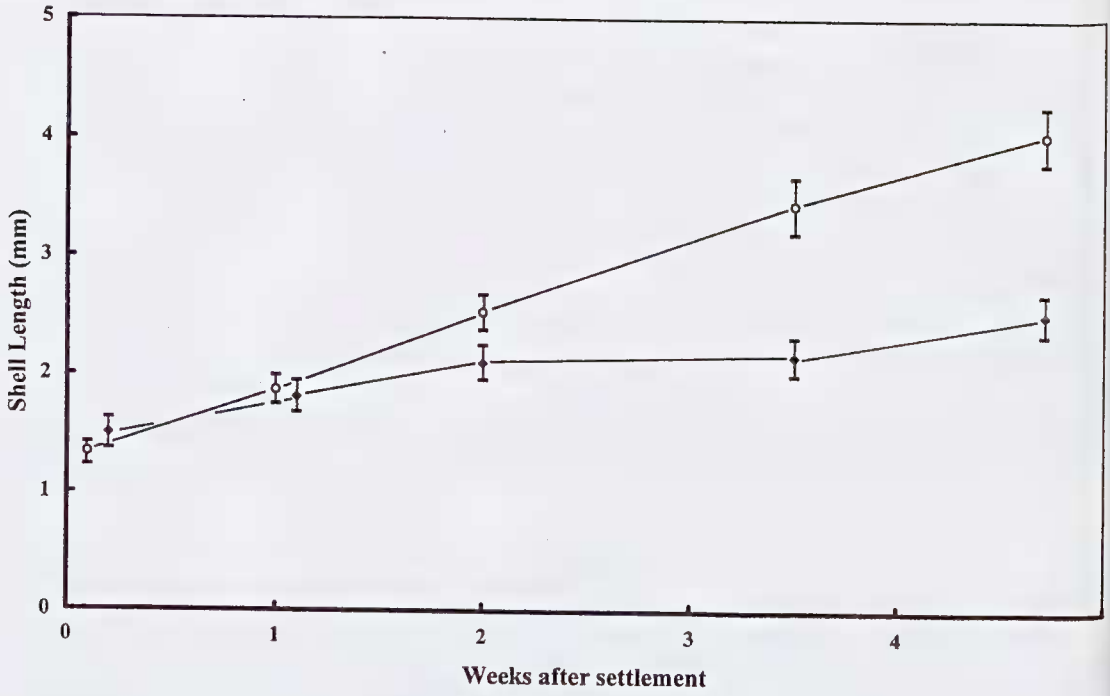

Figure 3. Growth of *Haliotis discus hannai* post-larvae (one month old) fed on CCA + diatoms (open circle) and CCA – diatoms (closed diamond). Data show mean shell length of post-larvae (± SE, n = 5-7) on each rock.

experimental period (85.0 ± 4.0 μm/day) and reached 4 mm in shell length at the end of the experiment after 33 days. In contrast, the mean growth rate of the individuals reared on CCA – diatoms (27.8 ± 3.3 μm/day) was significantly lower than that on CCA + diatoms throughout the experiment (t-test, $p<0.05$) and did not reach 3 mm. On CCA + diatoms, no dead individuals were observed during the experimental period whereas 2 out of 7 individuals died on CCA – diatoms by the end of the experiment.

The mean densities of diatom species on the rocks are shown in Table 1 and 2. Many diatoms were attached to CCA + diatoms rocks even after 1 week rearing of abalone. In contrast, diatom densities were very low throughout the experiment on CCA – diatoms rocks (Table 1, 2). Quantitative analyses of the feeding of post-larval abalone could not be carried out, but many diatom cells were found in the gut of post-larvae reared on CCA + diatoms whereas there were few cells in the gut of individuals on CCA – diatoms.

Table 3. Effect of rearing seawater with 6 mg/l concentration of GeO_2 on the metamorphosis and peristomal shell formation rates of larval abalone *Haliotis discus hannai*.

Rearing seawater	metamorphosis rate (%, mean ± SE)	peristomal shell formation rate (%, mean ± SE)
Filtered (0.45 μm) seawater	90.8 ± 1.0	63.4 ± 1.7
Seawater dissolved with GeO_2	94.6 ± 2.1	59.1 ± 5.7

Table 4. Effect of rearing seawater with 6 mg/l concentration of GeO_2 on the growth of post-larval abalone *Haliotis discus hannai*.

Rearing seawater	Initial shell length (μm, mean ± SE)	Daily growth rate (μm/day, mean ± SE)	No. of individuals measured	Rearing duration (days)
Filtered (0.45 μm) seawater	396.1 ± 13.9	37.0 ± 0.7	18	7
Seawater dissolved with GeO_2	394.3 ± 11.4	40.9 ± 0.7	18	7
Filtered (0.45 μm) seawater	847.7 ± 55.0	26.0 ± 5.9	5	15
Seawater dissolved with GeO_2	915.5 ± 63.1	31.7 ± 3.0	5	15
Filtered (0.45 μm) seawater	1899.7 ± 48.5	65.4 ± 7.8	6	7
Seawater dissolved with GeO_2	1972.0 ± 43.0	51.2 ± 9.3	6	7

The mean rates of metamorphosis and peristomal shell formation of larval abalone reared in GeO_2 seawater were not significantly different from those reared in filtered seawater (t-test, $p>0.05$; Table 3). There were no significant differences in the growth rates of all size groups of post-larvae between filtered seawater and GeO_2 seawater (t-test, $p>0.05$; Table 4). No dead individuals were observed in any size groups of abalone. These observations indicate that GeO_2 has no effect on larval metamorphosis, peristomal shell formation and post-larval growth.

Discussion

The results of this study indicate that diatoms are important food sources for the growth of post-larval *H. discus hannai* on CCA *L. yessoense*. The differences in the growth rate of post-larval abalone between CCA + diatoms and CCA – diatoms observed in the two experiments are considered to be due to the differences in amount of food sources which were derived from diatoms. We previously found that newly metamorphosed abalone which were fed on loosely adhered diatom species such as *Navicula ramosissima* (Agardh) Cleve 1895 and *Cylindrotheca closterium* (Ehrenberg) Reimann & Lewin 1964 were able to grow well up to a size of approximately 800 μm (Kawamura and Takami 1995). Extracellular mucus of diatoms was suggested as an primary food source for these early post-larvae (Kawamura and Takami 1995). In contrast, post-larvae over about 1 mm shell length require high levels of absorption of diatom cell contents in addition to any extracellular mucus for favorable growth (Kawamura *et al.* 1995). Since post-larvae cannot digest diatom cell contents in their alimentary canal without first rupturing the cell wall with the radula, only highly adhesive diatoms (such as *Cocconeis* spp.) and species with weakly silicified cell walls (such as *Cylindrotheca closterium*) which are easily broken open when they are grazed, are considered to be good food sources (Kawamura *et al.* 1995). In the present study, many diatoms including *Navicula* spp., *C. closterium* and *Cocconeis* spp. attached to the rocks used in CCA + diatoms treatment. Thus, CCA + diatoms rocks included the suitable food sources derived from diatoms for both younger and older post-larval abalone; extracellular mucus of diatoms for younger post-larvae and cell contents of *Cocconeis* spp. and *C. closterium* for older ones.

Although the survival rate of post-larvae on CCA – diatoms was significantly lower than that of individuals on CCA + diatoms at week 1(Fig. 2), post-larvae on CCA – diatoms grew as well as those on CCA + diatoms up to 1 week after settlement (Fig. 1). In addition, they reached 1 mm shell length 5 weeks after settlement (Fig. 1). It was reported that post-larval *H. rubra* can rasp the surface of CCA removing cytoplasmic content of the epithallium, suggesting that post-larvae depend for nutrition mostly on the polysaccharide layer grazed from CCA (Garland *et al.* 1985). We did not confirm whether post-larval *H. discus hannai* could remove the polysaccharide layer of the coralline alga or not. However, it is likely that post-larvae on CCA – diatoms showed comparable growth with those on CCA + diatoms rocks up to 1 week after settlement and grew to 1 mm at the end of

Experiment 1 using food sources associated with the CCA because there were few diatom cells attached to CCA – diatoms rocks (Table 1).

In the natural environment, diatom species with strongly adhesive solitary forms such as *Cocconeis* spp. were the dominant benthic diatoms in areas dominated by CCA, mainly due to their high tolerance to grazing pressures (Kawamura *et al.* 1992; Kawamura 1994). *Cocconeis* spp. are suitable food sources for post-larval *H. discus hannai* over approximately 1 mm shell length (Kawamura *et al.* 1995). Daume *et al.* (1997) also observed that post-larval *H. rubra* started to feed on *Cocconeis* spp. from 18 days after larval settlement even on a CCA, *Phymatolithon repandum* (Foslie) Wilks & Woelkerling. However, this diatom species is not considered a suitable food source for younger post-larvae (<800 μm) because of its relatively small quantities of extracellular substances (Kawamura and Takami 1995) and the difficulty of ingesting and absorbing diatom cell contents (Kawamura and Takami 1995; Daume *et al.* 1997). In this study, many loosely attaching diatom species which supply enough extracellular mucus for younger post-larvae were found growing on CCA + diatoms rocks as well as *Cocconeis* spp. The diatom communities growing on CCA in the natural environment may differ from those on CCA + diatoms rocks used in this study, because the occurrence of loosely attaching diatom species is limited in the natural environment (Kawamura *et al.* 1992). Thus, the supply of extracellular mucus of diatoms is probably lower in the natural environment than in the CCA + diatoms treatment. Other possible food sources on CCA in the natural environment include trail mucus of gastropods such as adult abalone (Takami *et al.* 1997), bacteria associated with CCA (Garland *et al.* 1985) or the mucus (Morse and Morse 1984), polysaccharide layer (Garland *et al.* 1985) of CCA themselves.

From the results of this study, it is considered that possible food materials derived from the CCA *L. yessoense* itself such as the polysaccharide layer are not sufficient food sources for the rapid growth of post-larval *H. discus hannai* from at least 500 μm shell length. In contrast, Shepherd and Daume (1996) reported that a CCA *Sporolithon durum* (Foslie) Townsend & Woelkerling 1995 had no biofilm on the surface, perhaps due to the sloughing of epithallial cells. They suggested that this CCA supplied enough food for *H. laevigata* and *H. scalaris* of 1 – 2 mm shell length. The dietary value of CCA themselves for post-larval abalone may differ between CCA species and their growth forms (Daume *et al.* 1997). Shepherd and Cannon (1988) reported that fragments of CCA were the predominant food item in the gut of *H. laevigata* and *H. scalaris* of 5 – 10 mm shell length and were eaten until at least 35 mm in their natural habitats. No individual abalone below 5 mm long were found to have macro-algae or calcareous fragments of CCA in their gut (Shepherd and Cannon 1988). *H. discus hannai* also ingests CCA and macro-algae from approximately 5 mm in shell length (Tomita and Tazawa 1971). A shift in the feeding habit from diatom feeding to macro-algal feeding may occur at about 5 mm shell length. Thus, it is possible that juvenile abalone can grow by feeding mainly on macro-algae including the fragments of CCA from this size. Further studies on the dietary value of CCA for the growth of juvenile abalone over 5 mm shell length are needed.

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